

Toward Understanding the Role of Amot80 Lipid Binding in Cellular Proliferation and Migration

Emily L. Donovan¹, Ann C. Kimble-Hill¹, Thomas D. Hurley¹, and Clark D. Wells¹

¹Department of Biochemistry and Molecular Biology, IU School of Medicine

Amots are adaptor proteins which coordinate signaling that controls cellular differentiation and proliferation, and their Amot coiled-coil homology (ACCH) domain is able to bind lipids with specificity which leads to membrane deformation and targets transcription factors to the nucleus. Understanding the biophysical mechanisms involved in lipid binding may provide pathways to modulate protein sorting and downstream signaling events inducing cellular differentiation, cancer cell proliferation, and migration. At this time, all work reported on signaling based on Amot expression is unable to distinguish between the role of the Amot80 and the 130 family members as they share a common ACCH domain. The goal of this project is to specifically relate the Amot80 ACCH lipid binding with function related to cancer phenotypes. Mutations were carried forward based on lipid sedimentation, FRET, and SAXS assays against the ACCH domain of the protein. Site-directed mutagenesis was then employed to probe the specific contributions of 7 selected lysines and arginines toward lipid head-group binding in the full length protein. The polarity/scaffolding signaling effect of mutations in the Amot80 will be monitored by matrigel, accumulation/cell counting, and titrated thymidine incorporation assays. Cell morphology will be imaged by confocal imaging, and cellular migration will be recorded by video. The effects on YAP1/2 and MAPK activation will be assessed by immunoblot analysis. The changes will then be correlated in extracellular scaffolding and migration with immunoblots and cellular staining. Likewise, effects on proliferation will be monitored by MTT assays. The hypothesis of this aim is that modulation of Amot's ability to bind selective lipids will interrupt the signaling pathways leading to cellular migration, differentiation, and proliferation. This work was supported by the IUPUI Undergraduate Research Opportunities Program (UROP) and NIH K01CA169078-01.

Mentor: Ann C. Kimble-Hill, Department of Biochemistry and Molecular Biology, IU of Medicine, Indianapolis, IN